

The Expression of Cyclooxygenase-2 and Survivin in Urinary Bladder Transitional Cell Carcinoma

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Background : The aim of this study was to investigate the expressions of cyclooxygenase-2 (COX-2) and survivin in bladder transitional cell carcinoma (TCC) that has different clinicopathologic characteristics, and we also wanted to determine if a relation exists between the COX-2 and survivin expressions. **Methods :** The expressions of COX-2 and survivin were investigated in 80 bladder TCCs by performing immunohistochemistry. **Results :** The normal bladder mucosa did not express COX-2 and survivin. COX-2 immunopositivity and cytoplasmic survivin immunopositivity were seen in 48% and 30% of bladder tumors, respectively. The expressions of COX-2 and survivin were closely related to the differentiation, depth and recurrence of bladder TCC, and there was a significant correlation in topographic distribution of COX-2 and survivin immunopositivity. In addition, COX-2 and survivin were predominantly expressed at the invasive front of tumors. **Conclusions :** This data suggest that COX-2 and survivin may be involved in the progression of bladder TCC, and there is a close correlation between the expressions of COX-2 and survivin.

Key Words : Bladder cancer; Cyclooxygenase-2; Survivin

Transitional cell carcinoma (TCC) of the urinary bladder accounts for about 3.2% of all the types of cancers and TCC is the seventh most common cancer worldwide with an estimated 260,000 new cases occurring each year in men and 76,000 new cases occurring in women.¹

The deregulation of apoptosis is a hallmark in human carcinogenesis, and bladder cancer has been shown to resist programmed cell death with the altered expression of both pro- and anti-apoptotic proteins.² Survivin is a member of a family of proteins called inhibitors of apoptosis protein (IAPs) and survivin is characterized by a highly conserved baculovirus IAP repeat domain. In contrast to the other members of the IAP protein family that are widely expressed in normal tissues,³ survivin is selectively overexpressed in common human malignancies, but it is barely detectable in normal differentiated adult tissues.⁴ In cancer cells, survivin is expressed during the G2/M phase of the cell cycle and it counteracts the induction of apoptosis during mitosis by interfering with the function of caspases.^{5,6} Its overexpression has been shown to be associated with an increased malignant potential and an unfavorable outcome in patients with various malignancies. In bladder TCC, a survivin expression has been demon-

strated by immunohistochemistry and it may be correlated with accelerated recurrences.⁷⁻⁹

Cyclooxygenase-2 (COX-2) is transiently induced by proinflammatory cytokines and growth factors, and COX-2 is involved in inflammation and mitogenesis.¹⁰ PGE₂ is related to carcinogenesis through immunosuppression, inhibiting apoptosis, increasing the metastatic potential of epithelial cells and promoting angiogenesis.^{11,12} A COX-2 overexpression was observed in various tumors as well as in bladder TCC.¹³⁻¹⁸ Moreover, a large case-control study demonstrated an overall decreased risk of bladder cancer in regular users of nonsteroidal anti-inflammatory drugs.¹⁹ In one nonsmall cell lung cancer cell line study, COX-2 has been shown to decrease the degradation of survivin, leading to its stabilization and the subsequent inhibition of apoptosis.²⁰ COX-2 was found to be overexpressed and positively correlated with a survivin expression in breast and endometrial cancer.^{21,22} The aim of this study was to investigate the expressions of COX-2 and survivin in bladder TCC that shows different clinicopathologic characteristics, and we also wanted to determine if a relation exists between the COX-2 and survivin expressions.

MATERIALS AND METHODS

Tissue samples and the patient population

We used eighty paraffin-embedded bladder TCCs and 5 normal bladder tissues obtained from the Department of Pathology at Dongguk University Kyongju Hospital. The cancer tissues were obtained from transurethral resection of bladder tumors. The normal bladder epithelial tissues were obtained from cases of chronic cystitis. The tumors were graded in accordance with the WHO-ISUP classification,²³ and the pathological T stage (pT, depth of invasion) was also determined. The age distribution of the patients whose tumors we were using was between 30 and 87 years, and the male to female ratio was 5.7:1. Disease recurrence was defined as any evidence of tumor in a retained bladder from 3 months after treatment.

Immunohistochemistry

Urinary bladder sections of 4 μm thickness were made and spread on poly-L-lysine coated slides. The paraffin sections were immersed in three changes of xylene and they were hydrated using a graded series of alcohol solutions. Antigen retrieval was routinely performed by immersing the sections in 0.01 M citrate buffer (pH 6.0) in a pressure cooker and performing auto-

claving for 15 min. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min and then the sections were incubated with primary antibody for 2 h at room temperature. The primary antibodies were rabbit polyclonal anti-COX-2 (Cayman Chemical, Ann Arbor, MI, USA, dilution 1:500) and anti-survivin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA 1:500). Staining was achieved with an EnVision kit (DAKO, Santa Barbara, CA, USA) and developed with 3, 3'-diaminobenzidine tetrahydrochloride (Zymed Laboratories, Inc., South San Francisco, CA, USA) as a chromogen. The sections were counterstained for 3 min with Meyer's hematoxylin and then they were mounted. As a negative control, rabbit IgG isotype were used instead of the primary antibody. The immunoreactivity for cytoplasmic COX-2 and survivin was evaluated by assessing the percentage of positive cells and this was graded as negativity (0-5%) or positivity (>5%). The nuclear survivin labeling index (LI) was calculated by counting at least 500 cells in 10 random high power fields.

Statistical analysis

Chi-square tests, Fisher's exact test, t-tests and one-way ANOVA were all used. Statistical significance was assumed if a p-value was less than 0.05. The data was expressed as means ± standard errors.

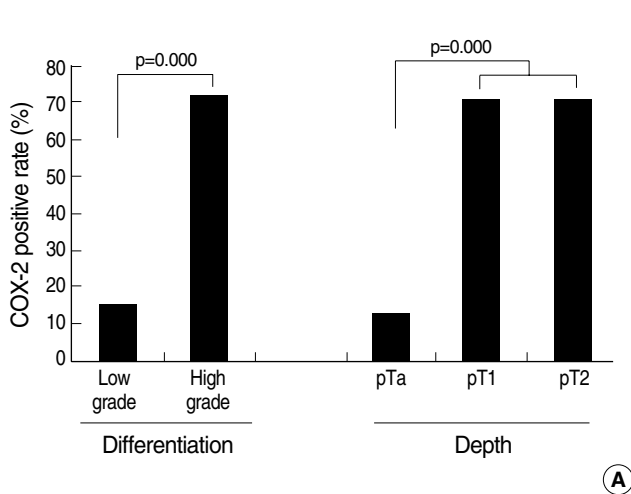
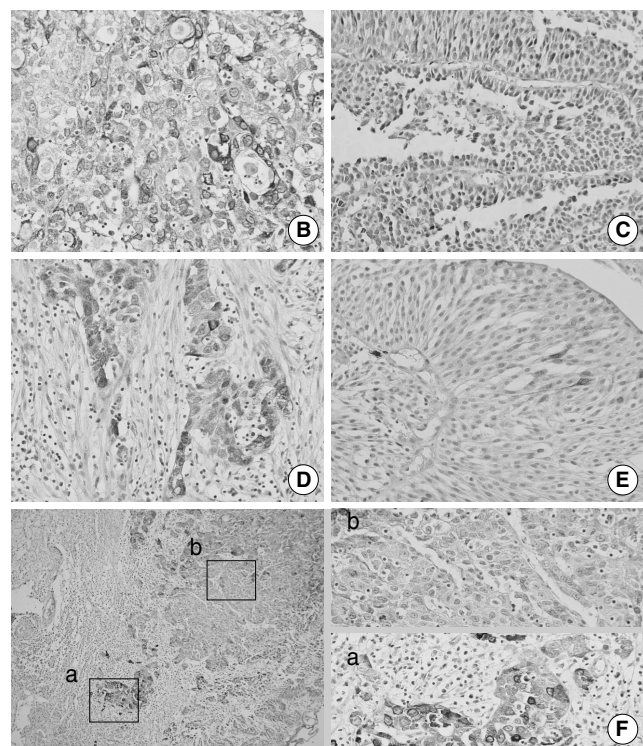


Fig. 1. Expression of COX-2 in bladder TCC by immunohistochemistry. COX-2 immunoreactivity is closely related to the differentiation and depth of tumor (p=0.000) (A). Bladder TCCs with high grade (B) and invasion (D) show strong immunoreactivity for COX-2 compared to bladder tumors with low grade (C) and noninvasion (E). COX-2 predominantly expresses at invasive front of tumors (a in F) compared to tumor center (b in F). COX-2, cyclooxygenase-2; TCC, transitional cell carcinoma.



RESULTS

Thirty two tumors were stage pTa, 24 were pT1 and 24 were pT2. Seventy percent patients (n=56) presented with superficial bladder tumors (pTa and pT1), while 30% (n=24) were invasive bladder cancers. Thirty four patients had low grade tumors, while 46 patients had high grade tumors. Twenty six patients had recurrent tumors. Tumors recurred in 32% (18/56) of superficial bladder cancers and in 33% (8/24) of the invasive ones. No significant relationship was present between the tumor depth and recurrence of tumor ($p=0.92$). High grade tumors recurred more frequently (37%, 17/46) than the low grade ones (26%, 9/34). However, there was no statistically significant difference for this ($p=0.32$).

Normal bladder mucosa did not express COX-2. COX-2 was mostly expressed in tumor cells and was focally expressed in stromal cells. Overall, COX-2 immunopositivity was seen in 48% (38/80) of the bladder tumors. As shown in Fig. 1A-E, the COX-2 expression was positive in 15% (5/34) of the low grade tumors, in 72% (33/46) of the high grade ones, and in

13% (4/32) of the pTa tumors, in 71% (17/24) of the pT1 and in 71% (17/24) of the pT2 tumors. Overall, COX-2 showed immunopositivity in 38% (21/56) of the superficial tumors and 71% (17/24) of the invasive ones. COX-2 immunoreactivity was closely related to the differentiation and depth of tumor ($p=0.000$). In addition, COX-2 was predominantly expressed at the invasive front of tumors (Fig. 1F).

The normal bladder mucosa did not express survivin. The cytoplasmic survivin immunopositivity was seen in 30% (24/80) of the bladder tumors. As shown in Fig. 2A, C and D, immunopositivity for cytoplasmic survivin was seen in 6% (2/34) of the low grade tumors, in 48% (22/46) of the high grade ones ($p=0.000$), in 19% (6/32) of the pTa tumors, in 17% (4/24) of the pT1 tumors and in 58% (14/24) of the pT2 tumors ($p=0.001$). Overall, immunopositivity for cytoplasmic survivin was seen in 18% (10/56) of the superficial tumors and in 58% (14/24) of the invasive ones. As shown in Fig. 2B, E and F, the nuclear survivin LI was 0.12 ± 0.03 in the low grade tumors, 0.39 ± 0.02 in the high grade ones ($p=0.000$), and 0.19 ± 0.03 in the pTa tumors, 0.30 ± 0.04 in the pT1 tumors and 0.36 ± 0.04 in the

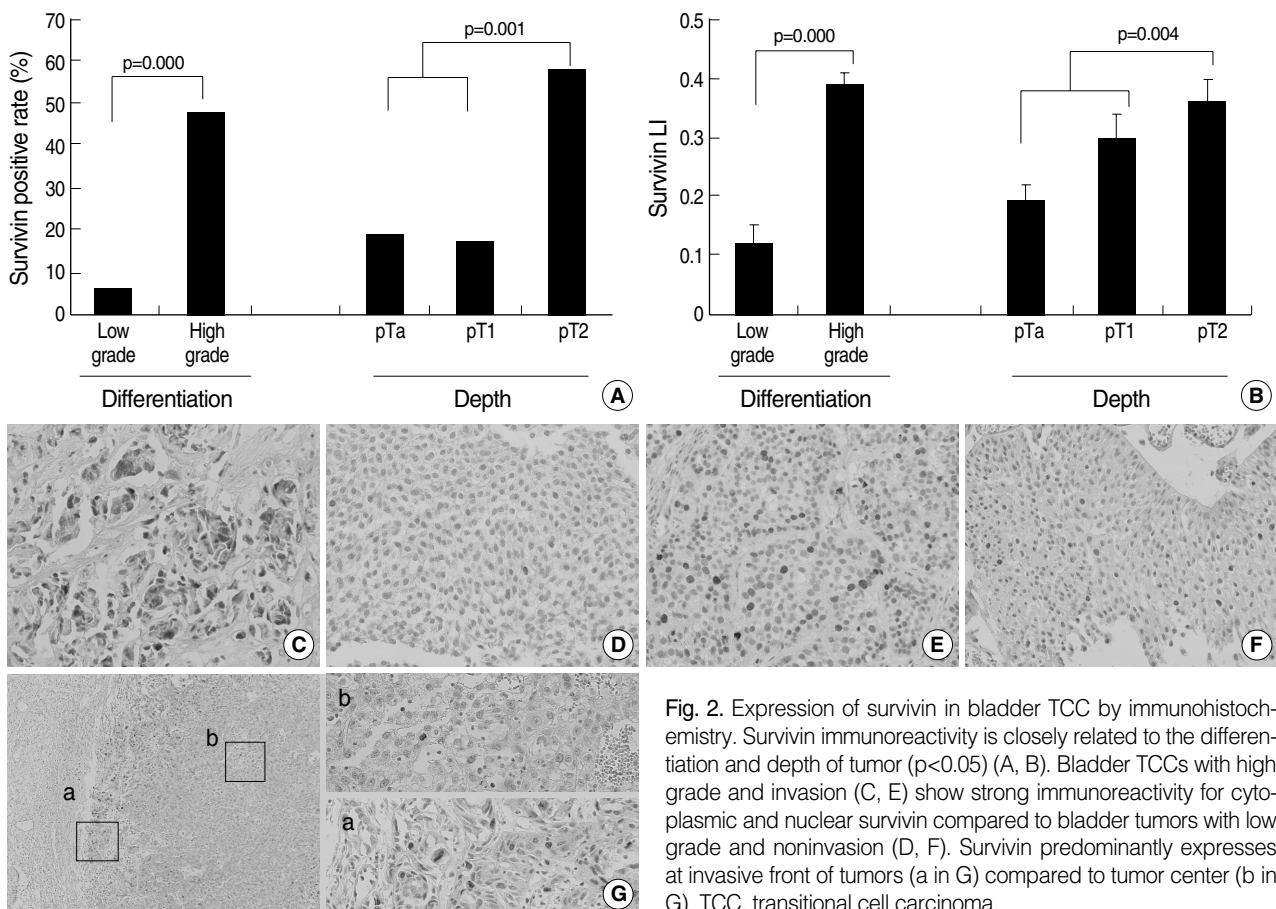


Fig. 2. Expression of survivin in bladder TCC by immunohistochemistry. Survivin immunoreactivity is closely related to the differentiation and depth of tumor ($p<0.05$) (A, B). Bladder TCCs with high grade and invasion (C, E) show strong immunoreactivity for cytoplasmic and nuclear survivin compared to bladder tumors with low grade and noninvasion (D, F). Survivin predominantly expresses at invasive front of tumors (a in G) compared to tumor center (b in G). TCC, transitional cell carcinoma.

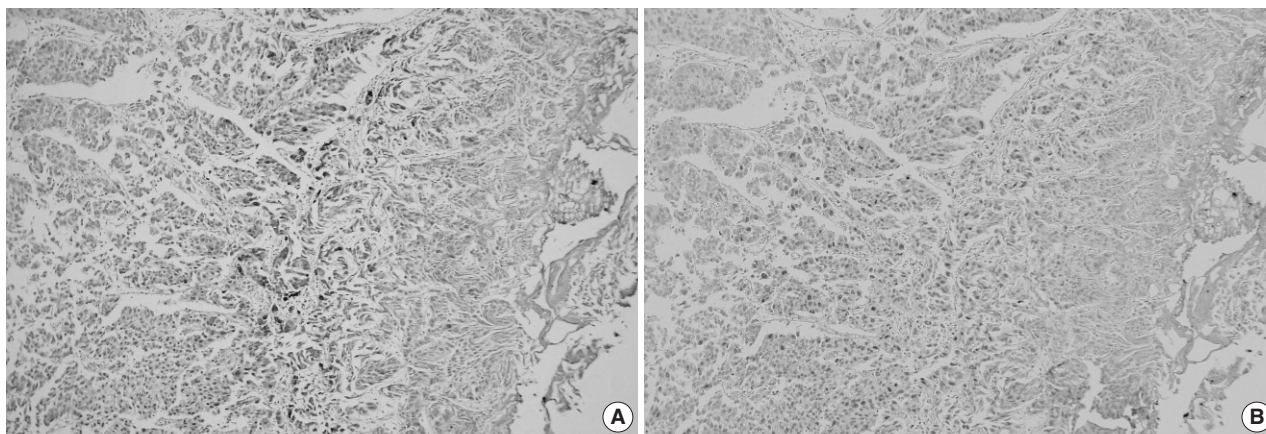


Fig. 3. Comparison of COX-2 and survivin expression at a same area by immunohistochemistry. Topographic distribution is similar between COX-2 (A) and nuclear survivin (B). COX-2, cyclooxygenase-2.

Table 1. Relationship between recurrence and survivin and COX-2 in bladder transitional cell carcinoma

		Survivin labeling index ^a	Cytoplasmic survivin positive rate (%)	COX-2 positive rate (%)
Recurrence	Absence	0.24 ± 0.02	14/54 (25.9)	25/54 (46.3)
	Presence	0.34 ± 0.04	10/26 (38.5)	13/26 (50.0)

^ap=0.038.
COX-2, cyclooxygenase-2.

pT2 tumors (p=0.004). Overall, the nuclear survivin LI was 0.23 ± 0.03 in the superficial tumors and 0.36 ± 0.04 in the invasive ones (p=0.008). The nuclear survivin expression was mainly seen at the invasive front of tumors (Fig. 2G).

As shown in Fig. 3, the topographic distribution was similar between COX-2 and nuclear survivin. Similarly, the nuclear survivin expression was significantly higher in the tumors with a positive COX-2 expression (0.35 ± 0.02) than in the tumors with a negative COX-2 expression (0.20 ± 0.03) (p=0.001).

We examined the relationship between tumor recurrence and the expression of COX-2 and survivin (Table 1). The nuclear survivin LI was significantly higher in the tumors with recurrence (0.34 ± 0.04) as compared with the tumors without recurrence (0.24 ± 0.02) (p=0.038). On consideration of tumor depth and differentiation, COX-2 immunopositivity was closely associated with recurrence in the tumors that were pTa and had a low grade (p=0.02). The rate of a positive COX-2 expression was 60% (3/5) in recurred cases and 6% (1/18) in non-recurred cases. Immunopositivity for cytoplasmic survivin was related to recurrence of tumors that were pT1 and had a high grade (p=0.001). The rate of a positive cytoplasmic survivin expression was 100% (9/9) in recurred cases and 0% (0/5) in non-

recurred cases.

DISCUSSION

We found that expressions of COX-2 and survivin were closely related to the differentiation, depth and recurrence of bladder TCC, and there was a significant correlation in the topographic distribution of COX-2 and survivin immunopositivity. Both COX-2 and survivin were predominantly expressed at the invasive front of tumors.

Contrary observations were reported in several previous studies that examined the role of a COX-2 expression on tumor differentiation and tumor depth. Shariata *et al.*¹⁴ reported cytoplasmic COX-2 immunoreactivity within the tumor cells, and found no relationship between the degree of the COX-2 expression and tumor differentiation. Several studies have reported that there was no relationship between tumor depth and the COX-2 expression.^{13,14} However, like our results, COX-2 immunopositivity was related to tumor differentiation and the tumor depth in recent studies.^{15,16} They have confirmed that COX-2 immunoreactivity was predominant in a subset population of infiltrating tumor cells.^{15,16} This finding was also observed in our study. Overall, COX-2 is suggested to have an important role during the progression of bladder TCC.

In superficial bladder TCC, Okajima *et al.*¹⁷ found more COX-2 protein positive samples in cases with recurrence than in cases without recurrence. Another study showed that the expression of COX-2 was correlated with recurrence and progression of pTa and grade III bladder tumors.¹⁸ Though the number of cases we examined was small, our results showed that COX-2 contributes

to tumor recurrence in cases with pTa and low grade. The patients with COX-2 positive superficial bladder cancer may need to be more vigorously followed up.

Like our results, several studies have demonstrated that survivin expression is closely related to the histological differentiation and the pathological stage of bladder TCC.^{8,9} Survivin was also reported to be a good marker to predict the recurrence of bladder TCC.^{8,24} Moreover, a recent study showed that a survivin expression decreased the 5-year overall survival rate.²⁵ Inhibition of the survivin expression and/or its function by dominant negative mutants, antisense cDNA or small interfering RNA constructs has been shown to inhibit tumor cell proliferation, and it markedly caused spontaneous, radiotherapy or chemotherapy-induced apoptosis.²⁶ Clinical trials targeting survivin might be expected in the near future.

We found that there was a correlation between the topographic distribution of COX-2 and survivin immunopositivity. COX-2 may be involved in bladder TCC carcinogenesis through increasing the activation of the anti-apoptotic protein survivin, though the cause and effect relationship could not be established in our study. However, some suggestions can be made from other available data. Survivin has been suggested to be as a target gene that is activated by the PI3K/Akt signaling pathway.²⁷ Pallares *et al.*²⁸ have reported that PTEN mutations may activate the PI3K/Akt signaling pathway and so interfere with apoptosis and cell proliferation by promoting a survivin expression. We know that COX-2 can activate the PI3K/Akt signaling pathway.²⁹ Hence, it is possible that COX-2 can promote survivin following this route. In one nonsmall cell lung cancer cell line study, COX-2 has been shown to decrease the degradation of survivin and this led to its stabilization and the subsequent inhibition of apoptosis.²⁰ Positive correlations between the expressions of COX-2 and survivin were recently reported in breast and endometrial cancers.^{21,22} Moreover, a recent study has demonstrated that survivin and COX-2 are co-expressed in human and mouse colon carcinoma and in terminally differentiated colonocytes.³⁰

In conclusion, our data suggest that COX-2 and survivin may be involved in the progression of bladder TCC, and there is a close correlation between the expressions of COX-2 and survivin.

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